Appl. No. 10/511.616 Election and Reply to Restriction to Office Action dated June 1, 2009

Atty. Docket No: 56029-51044

Amendments to the claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of claims:

- 1. (Currently amended) A live attenuated derivative of a pathogenic Salmonella species comprising consisting essentially of
- (a) a means for regulatable expression of a fur gene that encodes a regulatory protein, wherein a regulatable promotor is operably linked to said gene, wherein said gene is expressed when said attentuated strain is in the intestinal tract of an individual and said gene is not expressed when said attenuated strain is within internal tissues of an individual and wherein nonexpression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among Salmonella species and E. coli strains; and
- (b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among Salmonella species and E. coli strains;

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against Salmonella species and E. coli strains.

- 2. (Previously presented) The live attenuated derivative of claim 1, further comprising a means for non-expression of a serotype-specific antigen.
- 3. (Previously presented) The live attenuated derivative of claim 2, wherein said means for nonexpression of a serotype-specific antigen comprises a mutation in a gene selected from the group consisting of fliC and fliB.
- 4. (Previously presented) The live attenuated derivative of claim 3, wherein said mutation is a deletion mutation

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5. (Previously presented) The live attenuated derivative of claim 1, wherein said means of

regulatable expression comprises substituting the promoter of said gene that encodes a regulatory

protein with a regulatable promoter.

6. (Previously presented) The live attenuated derivative of claim 5 wherein said regulatable

promoter is the araCPBAD repressor-activator-promoter system.

7. (Canceled)

8. (Previously presented) The live attenuated derivative of claim 1 wherein said carbohydrate

antigen is an LPS O-antigen.

9. (Previously presented) The live attenuated derivative of claim 8 wherein said means for

regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigen.

10. (Previously presented) The live attenuated derivative of claim 9, wherein said means for

regulatable synthesis comprises a mutation in the pmi gene.

11. (Previously presented) A method for inducing an immune response sufficient for protection

against infection by Salmonella species and E. coli strains, said method comprising administering

to an individual the live attenuated derivative of claim 1.

12. (Currently amended) A live attenuated derivative of a pathogenic Salmonella species,

comprising consisting essentially of

(a) a means for regulatable expression of a fur gene, wherein the fur promoter is replaced

with a regulatable promotor operably linked to said fur gene, wherein said fur gene is expressed

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when said attentuated strain is in the intestinal tract of an individual and said fur gene is not expressed when said attenuated strain is within internal tissues of an individual; and

(b) a mutation that renders a pmi gene inoperable,

wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against Salmonella species and E. coli.

13. (Canceled)

14. (Previously presented) The live attenuated derivative of claim 12, wherein said means of (a) comprises replacing the fur promoter with the araCP_{BAD} activator-repressor-promoter system.

15. (Previously presented) The live attenuated derivative of claim 12 wherein said means of (a) comprises the ΔPfur::araCP_{BADfur} genetic construction.

16. (Previously presented) The live attenuated derivative of claim 12 wherein said mutation of (b) is a deletion mutation.

17. (Previously presented) A method of inducing a cross-protective immune response against Salmonella species, said method comprising administering to an individual the live attenuated derivative of claim 2.

18. (Canceled)

 (Currently amended) A vaccine comprising a live attenuated strain of Salmonella, wherein said live attenuated strain comprising consists essentially of

(a) a mutation in a pmi gene that renders said pmi gene non functional; and;

(b) a genetic construction that allows for regulatable expression of a fur gene, wherein said fur gene is expressed when said attenuated strain is in the intestinal tract of an individual and

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said fur gene is not expressed when said attenuated strain is within internal tissues of an individual and

wherein said vaccine has enhanced ability to stimulate cross protective immunity against Salmonella species and E. coli strains.

20. (Canceled)

- (Currently amended) A vaccine comprising a live attenuated strain of Salmonella, wherein said live attenuated strain comprising consists essentially of
 - (a) a mutation that renders a pmi gene non functional; and
- (b) a regulatable promotor operably linked to a fur gene wherein said fur gene is expressed when said attenuated strain is in the intestinal tract of an individual and said fur gene is not expressed when said attenuated strain is within internal tissues of an individual.
- (Previously presented) The vaccine of claim 21 wherein said regulatable promoter comprises the araCP_{BAD} activator-repressor-promoter system.
- 23. (Currently amended) A live attenuated derivative of a Salmonella species comprising consisting essentially of
- (a) a means for regulatable synthesis of LPS O-antigen side chains, wherein said Oantigen side chains are synthesized when said attenuated derivative is in the intestinal tract of an individual and are not synthesized when said attenuated derivative is within internal tissues of an individual: and
- (b) a means for regulatable expression of a fur gene, wherein said fur gene is expressed when said attenuated derivative is in the intestinal tract of an individual and wherein said fur gene is not expressed when said attenuated derivative $\underline{i}\underline{s}$ within internal tissues of an individual

wherein said attenuated derivative has increased ability to induce cross-protective immunity against infection by Salmonella species and E. coli strains.

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24. (Previously presented) The live attenuated derivative of claim 23 wherein said means for regulatable synthesis comprises a mutation in a gene that encodes a product necessary for

synthesis of LPS O-antigens.

25. (Previously presented) The live attenuated derivative of claim 24 wherein said gene that

encodes a product necessary for synthesis of LPS O-antigens is a pmi gene.

26. (Previously presented) The live attenuated derivative of claim 1, wherein said pathogenic Salmonella species is a Salmonella typhimurium comprising

(a) a ΔPfur::TTaraCP_{BAD}fur deletion-insertion mutation; and

(b) a Δpmi mutation.

27. (Canceled)

28. (Canceled)

29. (Canceled)

30. (Canceled)

31. (Currently amended) The live attenuated derivative of claim 1 [[30]], further comprising a

 Δpmi mutation.

32. (Currently amended) The live attenuated derivative of claim 1 comprising consisting

essentially of a ΔP fur:: $araCP_{BAD}$ fur genetic construction.

33. (Currently amended) A live attenuated derivative of a pathogenic Salmonella species

comprising consisting essentially of

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- (a) a means for regulatable expression of a <u>fur</u> gene that encodes a regulatory protein, wherein a regulatable promotor is operably linked to said gene, wherein said gene is expressed when said attentuated strain is in the intestinal tract of an individual and said gene is not expressed when said attenuated strain is within internal tissues of an individual and wherein non-expression of said regulatory protein in vivo causes synthesis of a first antigen that is conserved among Salmonella species and E. coli strains; and
- (b) a means for regulatable synthesis of a first carbohydrate antigen, wherein said first carbohydrate antigen ceases to be synthesized in vivo, exposing a second carbohydrate antigen that is conserved among Salmonella species and E. coli strains; and
- (c) a mutation of fliC or fliB, wherein said mutation results in deletion of the variable domain while retaining the N-terminal and C-terminal constant domains of flagellar proteins; wherein said attenuated derivative has enhanced ability to induce cross-protective immunity against Salmonella species and E. coli strains.
- 34. (Previously presented) The live attenuated derivative of claim 1, further comprising a means for biological containment.
- 35. (Previously presented) The live attenuated derivative of claim 34, wherein said means comprises a mutation that abolishes motility, prevents synthesis of the exopolysaccharide colanic acid, prevents synthesis of components of the bacterial extracellular matrix, reduces ability to withstand the stresses of stationary phase and starvation, reduces ability to use nucleic acids as a nutrient, or uncouples regulation of cellular activities from a dependence on protein synthesis.
- 36. (Previously presented) The live attenuated derivative of claim 35, wherein said mutation is selected from the group consisting of Δ(gmd-fcl)-26, ΔagfBAC811, ΔbcsABZC2118, ΔbcsEFG2319, ΔadrA1418, ΔmlrA34, ΔyhiR36::TT, ΔendA2311, ΔrelA1123.

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37. (Previously presented) The live attenuated derivative of claim 35, wherein said mutation consists of a mutation in a gene selected from the group consisting of gmd, fcl, agf, bcs, adr, mlr,

vhi, end and rel.

38. (Previously presented) The live attenuated derivative of claim 1, further comprising a

mutation in a gene selected from the group consisting of sip and sop.

39. (Previously presented) The live attenuated derivative of claim 38, wherein said mutation is

 $\Delta sop B1925$.

40. (Previously presented) The live attenuated derivative of claim 1, wherein said live

attenuated derivative comprises the $\Delta ilvG3$::TTaraCP_{BAD}lacI genetic construction.

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